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(FILE 'HOME' ENTERED AT 17:33:12 ON 19 JUL 2004)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 17:33:31 ON 19 JUL 2004

L1 699 S (CULTURED OR HUMAN)(W)SKIN(W)(EQUIVALENT OR SUBSTITUTE)
L2 2303535 S PROBLEM OR DIFFICULT? OR PITFALL OR DEFFICIEN?
L3 31 S L1 AND L2
L4 16 DUP REM L3 (15 DUPLICATES REMOVED)
L5 52 S SURFACE(W)ELECTRICAL(W)CAPACITANCE
L6 31 S L1 AND L2
L7 0 S L3 AND L5
L8 25 S L1 AND L5
L9 11 DUP REM L8 (14 DUPLICATES REMOVED)

=> d au ti so ab 1-11 l9

L9 ANSWER 1 OF 11 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AU Visscher M O (Reprint); Tolia G T; Wickett R R; Hoath S B
TI Effect of soaking and natural moisturizing factor on stratum corneum water-handling properties
SO JOURNAL OF COSMETIC SCIENCE, (MAY-JUN 2003) Vol. 54, No. 3, pp. 289-300. Publisher: SOC COSMETIC CHEMISTS, 120 WALL STREET, SUITE 2400, NEW YORK, NY 10005-4088 USA.
ISSN: 1525-7886.
AB Stratum corneum (SC) hydration is partially regulated by water-soluble molecules, natural moisturizing factor (NMF) that is associated with the corneocytes. Routine water exposure, e.g., bathing, may deplete NMF and alter the SC water-handling properties. We determined the effects of bathing and solvent extraction on the volar forearm skin of eleven healthy volunteers. Acetone/ether (A/E) was used to remove surface and upper SC lipids. Adjacent sites were soaked for ten minutes or treated with the A/E-plus-soak combination. Subsequently, an NMF formulation was applied to the treated sites, and transepidermal water loss (TEWL), hydration, and moisture accumulation rate (MAT) were measured. A/E extraction increased TEWL, but did not effect MAT. Soaking produced a short-term increase in TEWL, followed by a decrease, and substantially reduced MAT, an effect that was maintained for five hours. NMF application significantly decreased TEWL and significantly increased MAT for all sites. The replacement experiment suggests that the MAT reduction occurred as a result of extraction of hygroscopic NMF components. The effects of soaking and NMF application are more readily detected by the MAT technique, whereas TEWL is more sensitive to A/E extraction. The results support the use of multiple assessments of barrier function and raise questions about the effects of cumulative repeated water exposure on SC function.

L9 ANSWER 2 OF 11 MEDLINE on STN DUPLICATE 1
AU Boyce Steven T; Supp Andrew P; Swope Viki B; Warden Glenn D
TI Vitamin C regulates keratinocyte viability, epidermal barrier, and basement membrane in vitro, and reduces wound contraction after grafting of **cultured skin substitutes**.
SO Journal of investigative dermatology, (2002 Apr) 118 (4) 565-72. Journal code: 0426720. ISSN: 0022-202X.
AB **Cultured skin substitutes** have become useful as adjunctive treatments for excised, full-thickness burns, but no skin substitutes have the anatomy and physiology of native skin. Hypothetically, deficiencies of structure and function may result, in part, from nutritional deficiencies in culture media. To address this hypothesis, vitamin C was titrated at 0.0, 0.01, 0.1, and 1.0 mM in a **cultured skin substitute** model on filter inserts. **Cultured skin substitute** inserts were evaluated at 2 and 5 wk for viability by incorporation of 5-bromo-2'-deoxyuridine (BrdU) and by 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyl tetrazolium bromide (MTT) conversion. Subsequently, **cultured skin substitute** grafts consisting of cultured human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates were incubated for 5 wk in media containing 0.0 mM or 0.1 mM vitamin C, and then grafted to athymic mice. **Cultured skin substitutes** (n = 3 per group) were evaluated in vitro at 2 wk of incubation for collagen IV, collagen VII, and laminin 5, and through 5 wk for epidermal barrier by **surface electrical capacitance.** **Cultured skin substitutes** were grafted to full-thickness wounds in athymic mice (n = 8 per group), evaluated for **surface electrical capacitance** through 6 wk, and scored for percentage original wound area through 8 wk and for HLA-ABC-positive wounds at 8 wk after grafting. The data show that incubation of **cultured skin substitutes** in medium containing vitamin C results in greater viability (higher BrdU and MTT), more complete basement membrane development at 2 wk, and better epidermal barrier (lower **surface electrical capacitance**) at 5 wk in vitro. After grafting, **cultured skin substitutes** with vitamin C developed functional epidermal barrier earlier, had less wound contraction, and had more HLA-positive wounds at 8 wk than without vitamin C. These results suggest that incubation of **cultured skin substitutes** in medium containing vitamin C extends cellular viability, promotes formation of epidermal barrier in vitro, and promotes engraftment. Improved anatomy and physiology of **cultured skin substitutes** that result from nutritional factors in culture media may be expected to improve efficacy in treatment of full-thickness skin wounds.

L9 ANSWER 3 OF 11 MEDLINE on STN DUPLICATE 2

AU Supp A P; Wickett R R; Swope V B; Harriger M D; Hoath S B; Boyce S T

TI Incubation of **cultured skin substitutes** in reduced humidity promotes cornification in vitro and stable engraftment in athymic mice.

SO Wound repair and regeneration : official publication of the Wound Healing Society [and] European Tissue Repair Society, (1999 Jul-Aug) 7 (4) 226-37. Journal code: 9310939. ISSN: 1067-1927.

AB **Cultured skin substitutes** have been used successfully for adjunctive treatment of excised burns and chronic skin wounds. However, limitations inherent to all models of cultured skin include deficient barrier function in vitro, and delayed keratinization after grafting in comparison to native skin autografts. Experimental conditions for incubation of skin substitutes were tested to stimulate barrier development before grafting, and measure responses in function and stability after grafting. **Cultured skin substitutes** consisted of human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan biopolymer substrates. Parallel **cultured skin substitutes** were incubated at the air-liquid interface in ambient (48-61%) or saturated (79-91%) relative humidity, and grafted to athymic mice on culture day 14. Additional **cultured skin substitutes** were incubated in the experimental conditions for a total of 28 days. Cadaveric human skin and acellular biopolymer substrates served as controls. Epidermal barrier was evaluated as the change in surface hydration by **surface electrical capacitance** with the NOVA Dermal Phase Meter. **Cultured skin substitutes** and cadaveric skin incubated in ambient humidity had lower baseline **surface electrical capacitance** and less change in **surface electrical capacitance** than parallel samples incubated in saturated humidity at all time points in vitro. Data from healing **cultured skin substitutes** at 2, 4, 8 and 12 weeks after grafting showed an earlier return to hydration levels comparable to native human

skin, and more stable engraftment for skin substitutes from ambient humidity. The data indicate that **cultured skin substitutes** in ambient humidity have lower **surface electrical capacitance** and greater stability in vitro, and that they reform epidermal barrier more rapidly after grafting than **cultured skin substitutes** in saturated humidity. These results suggest that restoration of functional epidermis by **cultured skin substitutes** is stimulated by incubation in reduced humidity in vitro.

L9 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AU Boyce, S. T.; Swope, V.; Supp, A. P.; Warden, G. D.
TI Fibroblasts in **cultured skin substitutes** stimulate epidermal barrier and increase cellular viability.
SO Journal of Burn Care and Rehabilitation, (Jan.-Feb., 1999) Vol. 20, No. 1 PART 2, pp. S197. print.
Meeting Info.: 31st Annual Meeting of the American Burn Association. Lake Buena Vista, Florida, USA. March 24-27, 1999. American Burn Association.
CODEN: JBCRD2. ISSN: 0273-8481.

L9 ANSWER 5 OF 11 MEDLINE on STN DUPLICATE 3
AU Boyce S T; Supp A P; Swope V B; Warden G D
TI Topical sulfamylon reduces engraftment of **cultured skin substitutes** on athymic mice.
SO Journal of burn care & rehabilitation, (1999 Jan-Feb) 20 (1 Pt 1) 33-6.
Journal code: 8110188. ISSN: 0273-8481.
AB Sulfamylon (mafenide acetate) remains extremely valuable for the control of the bacterial contamination of burn wounds, but it is cytotoxic to cultured keratinocytes used for wound closure. Because composite skin substitutes develop a partial epidermal barrier in vitro, they may hypothetically tolerate the use of topical Sulfamylon. To test this hypothesis, **cultured skin substitutes** were prepared from cultured human fibroblasts; keratinocytes were attached to these collagen-based substrates, which were grafted to full-thickness wounds in athymic mice (n = 8 per group). Wounds were irrigated twice daily with 5% (wt/vol) Sulfamylon solution or with a formulation of noncytotoxic antimicrobials (0% Sulfamylon). On day 9 after grafting, the wounds were treated with dry dressings and assessed at 4 weeks for expression of human leukocyte antigens-A, B, C and at 2, 3, and 4 weeks for percentage of original wound area and **surface electrical capacitance** in picofarads (pF). Data were analyzed for statistical significance ($P < .05$) by Fisher's exact test, Student's t test, and repeated measures analysis of variance: [table: see text] The data demonstrate that irrigation of **cultured skin substitutes** with a solution of 5% Sulfamylon results in smaller wound area, fewer wounds that contain human cells, and greater surface hydration (higher **surface electrical capacitance**) than irrigation with noncytotoxic antimicrobial agents. These results support the conclusion that **cultured skin substitutes** of this type do not tolerate the chemical toxicity of Sulfamylon as well as skin autografts. Further improvements in the properties of the epidermal barrier of **cultured skin substitutes** may facilitate the use of Sulfamylon or other potent antimicrobial agents for the management of microbial contamination during engraftment of transplanted skin cells.

L9 ANSWER 6 OF 11 MEDLINE on STN DUPLICATE 4
AU Boyce S T
TI Skin substitutes from cultured cells and collagen-GAG polymers.
SO Medical & biological engineering & computing, (1998 Nov) 36 (6) 791-800.
Ref: 101
Journal code: 7704869. ISSN: 0140-0118.
AB Engineering skin substitutes provides a potential source of advanced therapies for the treatment of acute and chronic wounds. **Cultured**

skin substitutes (CSS) consisting of human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates have been designed and tested in preclinical and clinical studies. Cell culture techniques follow general principles of primary culture and cryopreservation in liquid nitrogen for long-term storage. Biopolymer substrates are fabricated from xenogeneic (bovine) collagen and glycosaminoglycan that are lyophilised for storage until use. At maturity in air-exposed culture, CSS develop an epidermal barrier that is not statistically different from native human skin, as measured by **surface electrical capacitance**. Preclinical studies in athymic mice show rapid healing, expression of cytokines and regulation of pigmentation. Clinical studies in burn patients demonstrate a qualitative outcome with autologous skin that is not different from 1:4 meshed, split-thickness autograft skin, and with a quantitative advantage over autograft skin in the ratio of healed skin to biopsy areas. Chronic wounds resulting from diabetes or venous stasis have been closed successfully with allogeneic CSS prepared from cryopreserved skin cells. These results define the therapeutic benefits of **cultured skin substitutes** prepared with skin cells from the patient or from cadaver donors. Future directions include genetic modification of transplanted cells to improve wound healing transiently or to deliver gene products systemically.

L9 ANSWER 7 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AU Boyce, S. T. [Reprint author]; Swope, V. B.; Supp, A. P.; Warden, G. D.
TI Vitamin C promotes epidermal barrier and DNA synthesis in keratinocytes of **cultured skin substitutes**.
SO Molecular Biology of the Cell, (Nov., 1997) Vol. 8, No. SUPPL., pp. 339A. print.
Meeting Info.: 37th Annual Meeting of the American Society for Cell Biology. Washington, D.C., USA. December 13-17, 1997. American Society for Cell Biology.
CODEN: MBCEEV. ISSN: 1059-1524.

L9 ANSWER 8 OF 11 MEDLINE on STN DUPLICATE 5
AU Harriger M D; Supp A P; Swope V B; Boyce S T
TI Reduced engraftment and wound closure of cryopreserved **cultured skin substitutes** grafted to athymic mice.
SO Cryobiology, (1997 Sep) 35 (2) 132-42.
Journal code: 0006252. ISSN: 0011-2240.
AB Cryopreservation of **cultured skin substitutes** is a requirement for establishment of banks of alternative materials for treatment of acute and chronic skin wounds. To determine whether cryopreservation of skin substitutes that contain cultured cells reduces their efficacy for wound closure, cell-biopolymer grafts were frozen, recovered into culture, and grafted to wounds on athymic mice. Grafts consisted of cultured human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates that were frozen in cell culture medium with 20% serum and 10% DMSO at a controlled rate and stored overnight in liquid nitrogen. After recovery into culture for 24 h, frozen or unfrozen (control) skin substitutes were grafted to full-thickness wounds on athymic mice. Wound area and **surface electrical capacitance** were measured at 2, 3, and 4 weeks after grafting at which time animals were sacrificed. Wounds were scored for presence of human cells by direct immunofluorescence staining with a monoclonal antibody to HLA-ABC. The data demonstrate that cell-biopolymer grafts are less efficacious after controlled-rate cryopreservation using 10% DMSO as a cryoprotectant. Frozen grafts at 4 weeks after surgery have significantly smaller wound areas, higher capacitance (wetter surface), and fewer healed wounds that contain human cells. The results suggest that these conditions for cryopreservation of cultured grafts reduce graft viability. Improved conditions for cryopreservation are required to maintain viability and efficacy of **cultured skin substitutes** after frozen

storage.
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L9 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 6
AU Boyce S T; Supp A P; Harriger M D; Pickens W L; Wickett R R; Hoath S B
TI **Surface electrical capacitance** as a noninvasive index of epidermal barrier in **cultured skin substitutes** in athymic mice.
SO Journal of investigative dermatology, (1996 Jul) 107 (1) 82-7. Journal code: 0426720. ISSN: 0022-202X.
AB Restoration of an epidermal barrier is a definitive requirement for wound closure. To determine formation of an epidermal barrier as a function of hydration of the stratum corneum, we measured **surface electrical capacitance** (SEC) of the epidermis in **cultured skin substitutes** (CSS) in vitro and after grafting to athymic mice. CSS were prepared from human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates. On culture days 3, 7, 14, 17, and 21, SEC was measured in situ. CSS (n = 18; mean +/- SEM) showed a time-dependent decrease of SEC (picoFarads, "pF") from 4721 +/- 28 pF on day 3 to 394 +/- 117 pF on day 14, and subsequent increase to 1677 +/- 325 pF on day 21. After 14-d incubation, parallel CSS samples (n = 5) or murine autografts (n = 5) were grafted orthotopically to athymic mice. After grafting, CSS showed decreases in SEC from 910 +/- 315 pF at 2 wk to 40 +/- 10 pF at 4 wk with no significant decreases thereafter. Control values for murine autograft were 870 +/- 245 pF at 2 wk, and 87 +/- 30 pF at 4 wk. SEC values for native murine skin (n = 10) were 91 +/- 18 pF, and for native human skin (n = 10) were 32 +/- 5 pF. The data demonstrate that SEC decreases with time in culture and that healed or intact skin has approximately 10- to 100-fold lower SEC than CSS in vitro. This noninvasive technique provides a quantitative index of epidermal barrier in CSS in vitro and demonstrates the development of functional epidermal barrier during healing of wounds treated with **cultured skin substitutes**.

L9 ANSWER 10 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AU Goretsky, Michael J.; Supp, Andrew P.; Greenhalgh, David G.; Warden, Glenn D.; Boyce, Steven T. [Reprint author]
TI **Surface electrical capacitance** as an index of epidermal barrier properties of composite skin substitutes and skin autografts.
SO Wound Repair and Regeneration, (1995) Vol. 3, No. 4, pp. 419-425. ISSN: 1067-1927.
AB Restoration of the epidermal barrier is a requirement for burn wound closure, A rapid, reliable, and noninvasive measure of the rate of restoration of the epidermal barrier is not readily available. To monitor the reformation of the epidermal barrier, we measured **surface electrical capacitance** on **cultured skin substitutes** (human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates) and split-thickness skin autografts grafted to patients. Data were collected from four patients with burns and one pediatric patient with a congenital hairy nevus comprising gt 60% total body surface area. Capacitance measurements were performed at days 7, 10, 12, 14, and 28 by direct contact of the capacitance probe for 10 seconds to the **cultured skin substitutes** or split-thickness autograft. On postoperative days 7, 10, 12,14, 21, and 28, the **surface electrical capacitance** of **cultured skin substitutes** after 10 seconds of sampling was 2468 +- 268, 1443 +- 439, 129 +- 43, 200 +- 44, 88 +- 20, and 74 +- 19 picofarads (mean +- standard error of the mean), respectively. **Surface electrical capacitance** for split-thickness autograft on the same days was 1699 +- 371, 1914 +- 433, 125 +- 16, 175 +- 63,110 +- 26, 271 +- 77 picofarads, respectively. **Surface electrical capacitance** in all of the grafts decreased

with time, **Cultured skin substitutes** had approximately the same 10-second capacitance values as split-thickness autograft during 3 weeks of healing and approached values for uninjured skin (32 +- 5 picofarads) by 12 days. Measurement of **surface electrical capacitance** is a direct, inexpensive, and convenient index for noninvasive monitoring of epidermal barrier formation.

L9 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7
AU Boyce, S. T. [Reprint author]; Supp, A. P.; Pickens, W. L.; Hoath, S. B.
TI **Surface electrical capacitance** as a non-invasive measure of epidermal barrier of **cultured skin substitutes** in vitro and in vivo.
SO Journal of Investigative Dermatology, (1994) Vol. 102, No. 4, pp. 537.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology. Baltimore, Maryland, USA. April 27-30, 1994.
CODEN: JIDEAE. ISSN: 0022-202X.

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